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| <p>(54) Title: INJECTABLE HYDROGEL COMPOSITIONS</p> | | |
| <p>(57) Abstract</p> | | |
| <p>Slowly polymerizing hydrogels are provided which are useful as a means of delivering large numbers of isolated cells via injection. The gels promote engraftment and provide three-dimensional templates for new cell growth. The resulting tissue is similar in composition and histology to naturally occurring tissue. This method can be used for a variety of reconstructive procedures, including custom molding of cell implants to reconstruct three-dimensional tissue defects, as well as implantation of tissues generally. The polymers permit construction of muscle and cartilage tissues which are useful to repair defects such as reflux and incontinence.</p> | | |

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INJECTABLE HYDROGEL COMPOSITIONS

Background of the Invention

The present invention is generally in the area of using polymeric hydrogel-cell compositions in medical treatments.

5 Craniofacial contour deformities

Craniofacial contour deformities, whether traumatic, congenital, or aesthetic, currently require invasive surgical techniques for correction. Furthermore, deformities requiring augmentation often necessitate the use of alloplastic prostheses which suffer from problems of infection and
10 extrusion. A minimally invasive method of delivering additional autogenous cartilage or bone to the craniofacial skeleton would minimize surgical trauma and eliminate the need for alloplastic prostheses. If one could transplant via injection and cause to engraft large numbers of isolated cells, one could augment the craniofacial osteo-cartilaginous
15 skeleton with autogenous tissue, but without extensive surgery.

Unfortunately, attempts to inject dissociated cells subcutaneously or to implant dissociated tissues within areas of the body such as the peritoneum have not been successful. Cells are relatively quickly removed, presumably by phagocytosis and cell death.

20 Cells can be implanted onto a polymeric matrix and implanted to form a cartilaginous structure, as described in U.S. Patent No. 5,041,138 to Vacanti, et al., but this requires surgical implantation of the matrix and shaping of the matrix prior to implantation to form a desired anatomical structure.

25 Vesicoureteral reflux.

Vesicoureteral reflux is a condition wherein there is an abnormal development of the ureteral bud as it enters the bladder during
embryologic development. The shortened course of the ureter through the bladder musculature decreases the ureteral resistance and allows for urine
30 to reflux from the bladder reservoir back up into the ureter and into the kidney. With this condition, bacteria which may occasionally be present in the bladder through retrograde urethral transport, can reach the kidneys

and cause recurrent pyelonephritis. In addition, the constant back pressure of the urine into the calyces and renal pyramids results in mechanical damage to the renal parenchyma. If untreated, urinary vesicoureteral reflux can cause loss of renal parenchyma, and in some instances, renal failure, as reviewed by Atala and Casale, Infections in Urology 39-43 (March/April 1990). In 1960, 70% of the patients with renal failure were described as having vesicoureteral reflux as the primary etiology. With the advent of new diagnostic and treatment modalities, patients with vesicoureteral reflux now account for less than 1% of the renal failure population.

In the past, vesicoureteral reflux was usually diagnosed with a voiding cystogram after the child presented with repeated episodes of pyelonephritis. With the increased use of prenatal and postnatal sonography, hydronephrosis is more detectable, prompting further radiologic workup and earlier detection, as reported by Atala and Casale. Vesicoureteral reflux is graded depending on the severity. Grade 1 reflux signifies that urine is seen refluxing from the bladder up to the ureter only; in grade 2 reflux, urine refluxes into the ureter and calyceal dilatation. Grade 4 and 5 reflux are more severe, showing ureteral tortuosity and further calyceal blunting and dilatation, respectively.

The treatment of vesicoureteral reflux has been well established over the last decade. Initially it was believed that all patients with reflux would require surgery. Another school of management soon proposed that only medical therapy with antibiotics was required. It is now well established that the treatment of reflux depends on many factors, including the severity of reflux, associated congenital abnormalities, and the social situation of the child (parental compliance with medical treatment). Medical treatment is usually recommended for patients with grade 1 and 2 reflux, which usually resolve on their own as the bladder/ureteral configuration changes with growth. Grade 3 reflux is generally managed with medical therapy unless it persists or breakthrough

infections occur while on antibiotic suppression. Surgical treatment is usually required for grade 4 and 5 reflux.

Medical treatment implies that the patient is treated with daily suppressive antibiotics. A close follow-up is required in these patients, generally consisting of a catheterized urine culture every three months, an ultrasound exam and serum analysis every six months, a fluoroscopic or nuclear voiding cystourethrogram every year, and a DMSA renal scan every two years. Surgical treatment consists of an open surgery wherein a low abdominal incision is made, the bladder is entered, the ureters are mobilized and new ureteral submucosal tunnels are created; thereby extending the muscular backing of the ureter which increases their resistance. These patients require a general endotracheal anesthetic for a four to five hour surgery, an epidural catheter for both intraoperative and postoperative pain control, a bladder catheter for drainage, a perivesical drain, and a five to six day hospital stay. Antibiotic therapy and bladder antispasmodics are required post-operatively.

Although open surgical procedures for the correction of reflux have excellent results in the hands of experienced surgeons, it is associated with a well recognized morbidity, including pain and immobilization of a lower abdominal incision, bladder spasms, hematuria, and post-operative voiding frequency in some children. In an effort to avoid open surgical intervention, widespread interest was initiated by Matouschek's clinical experience with the endoscopic injection of Teflon™ (polytetrafluoroethylene) paste subureterally in 1984, as reported in Matouschek, E.: Die Behandlung des vesikorenenalen Refluxes durch transueterale Einspritzung von polytetrafluoroethylenepast. Urologe, 20:263 (1981). With this technique, a cystoscope is inserted into the bladders, a needle is inserted through the cystoscope and placed under direct vision underneath the refluxing ureter in the submucosal space, and Teflon™ paste is injected until the gaping ureteric orifice configuration changes into a half-moon slit. The Teflon™ paste, injected endoscopically, corrects the reflux by acting as a bulking material which

increases ureteral resistance. However, soon after the introduction of this treatment, a controversy regarding the use of Teflon™ paste ensued.

Malizia et al. "Migration and granulomatous reaction after periurethral injection of polymer (polytetrafluoroethylene)" JAMA, 251:3277 (1984), showed granuloma formation and particulate migration to the brain, lungs, and lymph nodes in animal studies. Polytetrafluoroethylene migration and granuloma formation have also been reported in humans by Claes et al., "Pulmonary migration following periurethral polytetrafluoroethylene injection for urinary incontinence" J. Urol., 142:821 (1989). The safety of Teflon™ for human use was questioned, and the paste was thereafter banned by the FDA.

However, there are definite advantages in treating vesicoureteral reflux endoscopically. The method is simple and can be completed in less than fifteen minutes, it has a success rate of greater than 85% with low morbidity and it can be performed in an outpatient basis, as reported by Atala et al, "Endoscopic treatment of vesicoureteral reflux with a self-detachable balloon system" J. Urol. 148:724 (1992). The goal of several investigators has been to find alternate implant materials which would be safe for human use.

Bovine dermal collagen preparations have been used to treat reflux endoscopically. However, only 58.5% of the patients were cured at one year follow-up, as described by Leonard et al, "Endoscopic injection of glutaraldehyde cross-linked bovine dermal collagen for correction of vesicoureteral reflux" J. Urol. 145:115 (1991). The collagen implant volume decreases with time, which results in high percentage of recurrence of reflux, over 90% within 3 years. The high failure rate with this substance presents a high risk to the unaware patient of developing renal damage after treatment.

A paste consisting of textured microparticles of silicone, suspended in a hydrogel, has been injected subureterally to correct reflux with an initial success rate of 91% in one European study, as reported by Buckley et al., "Endoscopic correction of vesicoureteric reflux with injectable

silicone microparticles" J. Urol. 149: 259A (1993). However, distant particle migration has been observed in animal models, as reported by Henly et al., "Particulate silicone for use in periurethral injections: a study of local tissue effects and a search for migration" J. Urol. 147:376A (1992). Approximately thirty percent of the silicone particles have a diameter which is less than 100 μ m. This suggests that thirty percent of the silicone particles have a potential for distant organ migration through the macrophage system. The manufacturer of this technology tried unsuccessfully to obtain FDA approval, and subsequently filed for bankruptcy.

Laparoscopic correction of reflux has been attempted in both an animal model (Atala et al, "Laparoscopic correction of vesicoureteral reflux" J. Urol. 150:748 (1993)) and humans (Atala, "Laparoscopic treatment of vesicoureteral reflux" Dial Ped Urol 14:212 (1993)) and is technically feasible. However, at least two surgeons with laparoscopic expertise are needed, the length of the procedure is much longer than with open surgery, the surgery is converted from an extraperitoneal to an intraperitoneal approach, and the cost is higher due to both increased operative time and the expense of the disposable laparoscopic equipment.

Despite the fact that over a decade has transpired since the Teflon™ controversy, little progress has been made in this area of research. The ideal substance for the endoscopic treatment of reflux should be injectable, non-antigenic, non-migratory, volume stable, and safe for human use (Atala et al, 1992).

Urinary incontinence.

Urinary Incontinence is the most common and the most intractable of all GU maladies. Urinary incontinence, or the inability to retain urine and not void urine involuntarily, is dependent on the interaction of two sets of muscles. One is the detrusor muscle, a complex of longitudinal fibers forming the external muscular coating of the bladder. The detrusor is activated by parasympathetic nerves. The second muscle is the smooth/striated muscle of the bladder sphincter. The act of voiding

requires the sphincter muscle be voluntarily relaxed at the same time that the detrusor muscle of the bladder contracts. As a person ages, his ability to voluntarily control the sphincter muscle is lost in the same way that general muscle tone deteriorates with age. This can also occur when a radical event such as paraplegia "disconnects" the parasympathetic nervous system causing a loss of sphincter control. In different patients, urinary incontinence exhibits different levels of severity and is classified accordingly.

The most common incontinence, particular in the elderly, is urge incontinence. This type of incontinence is characterized by an extremely brief warning following by immediate urination. This type of incontinence is caused by a hyperactive detrusor and is usually treated with "toilet training" or medication. Reflex incontinence, on the other hand, exhibits no warning and is usually the result of an impairment of the parasympathetic nerve system such as a spinal cord injury.

Stress incontinence is most common in elderly women but can be found in women of any age. It is also commonly seen in pregnant women. This type of incontinence accounts for over half of the total number of cases. It is also found in men but at a lower incidence. Stress incontinence is characterized by urine leaking under conditions of stress such as sneezing, laughing or physical effort. There are five recognized categories of severity of stress incontinence, designated as types 0, 1, 2a, 2b, and 3. Type 3 is the most severe and requires a diagnosis of intrinsic Sphincter Deficiency or ISD (Contemporary Urology, March 1993). There are many popular treatments including weight loss, exercise, medication and in more extreme cases, surgical intervention. The two most common surgical procedures involve either elevating the bladder neck to counteract leakage or constructing a lining from the patient's own body tissue or a prosthetic material such as PTFE to put pressure on the urethra. Another option is to use prosthetic devices such as artificial sphincters to external devices such as intravaginal balloons or penile clamps. For treatment of type 3 stress incontinence, there has been

a recent trend toward injection of Teflon™ or collagen paste around the sphincter muscle in order to "beef up" the area and improve muscle tone. None of the above methods of treatment, however, are very effective for periods in excess of a year.

5 Overflow incontinence is caused by anatomical obstructions in the bladder or underactive detrusors. It is characterized by a distended bladder which leads to frequent urine leakage. This type of incontinence is treated acutely by catheterization and long-term by drug therapy. Enuresis or bed-wetting is a problem in pediatrics and is controlled by
10 various alarming devices and pads with sensors. Enuresis is not considered a serious problem unless it lasts beyond the age of four or five. Finally, there is true functional incontinence which occurs in patients with chronic impairment either of mobility or mental function. Such patients are usually treated by the use of diapers, incontinence pads
15 or continuous catheterization (BBI, 1985 Report 7062).

 WO 94/25080 describes the use of injectable polysaccharide-cell compositions for delivering isolated cells by injection. There is a need for improved injectable polymer-cell compositions which are biocompatible and biodegradable for delivering isolated cells by injection
20 or implantation.

 Accordingly, it is an object of the present invention to provide methods and compositions for injection of cells to form cellular tissues and cartilaginous structures.

 It is a further object of the invention to provide improved
25 compositions to form cellular tissues and cartilaginous structures including non-cellular material which will degrade and be removed to leave tissue or cartilage that is histologically and chemically the same as naturally produced tissue or cartilage.

 It is another object of the present invention to provide improved
30 methods and materials for treating vesicoureteral reflux, incontinence, and other defects which results in a natural and permanent cure to the defect.

It is a further object of the present invention to provide methods and materials for treating vesicoureteral reflux, incontinence, and other defects which is quick, simple, safe, and relatively non-invasive.

Summary of the Invention

5 Slowly polymerizing, biocompatible, biodegradable hydrogels are provided which are useful for delivering large numbers of isolated cells into a patient to create an organ equivalent or tissue such as cartilage. The gels promote engraftment and provide three dimensional templates for new cell growth. The resulting tissue is similar in composition and
10 histology to naturally occurring tissue. In one embodiment, cells are suspended in a polymer solution and injected directly into a site in a patient, where the polymer crosslinks to form a hydrogel matrix having cells dispersed therein. In a second embodiment, cells are suspended in a polymer solution which is poured or injected into a mold having a desired
15 anatomical shape, then crosslinked to form a hydrogel matrix having cells dispersed therein which can be implanted into a patient. Ultimately, the hydrogel degrades, leaving only the resulting tissue.

 This method can be used for a variety of reconstructive procedures, including custom molding of cell implants to reconstruct three
20 dimensional tissue defects, as well as implantation of tissues generally.

 In another embodiment, a method of treatment of vesicoureteral reflux, incontinence and other defects is provided wherein bladder muscle cells are mixed with a liquid polymeric material, to form a cell
 suspension, which is injected into the area of the defect, in an amount
25 effective to yield a tissue that corrects the defect, for example, which provides the required control over the passage of urine. In one embodiment, human bladder muscle specimens or chondrocytes are obtained and processed, the cells are mixed with the polymer, which is designed to solidify at a controlled rate when contacted with a
30 crosslinking agent, and then the cells are injected at the desired site where they proliferate and correct the defect.

Detailed Description of the Invention

Techniques of tissue engineering employing biocompatible polymer scaffolds hold promise as a means of creating alternatives to prosthetic materials currently used in craniomaxillofacial surgery, as well as formation of organ equivalents to replaced diseased, defective, or injured tissues. However, polymers used to create these scaffolds, such as polylactic acid, polyorthoesters, and polyanhydrides, are difficult to mold and hydrophobic, resulting in poor cell attachment. Moreover, all manipulations of the polymers must be performed prior to implantation of the polymeric material.

Biocompatible polymers described herein such as polysaccharides can form hydrogels which are malleable and can be used to encapsulate cells. To form a hydrogel containing the cells, a polymer solution is mixed with the cells to be implanted to form a suspension. Then, in one embodiment, the suspension is injected directly into a patient prior to crosslinking of the polymer to form the hydrogel containing the cells. The hydrogel forms over a short period of time. In a second embodiment, the suspension is injected or poured into a mold, where it crosslinks to form a hydrogel of the desired anatomical shape having cells dispersed therein which then may be implanted in a patient. The hydrogel may be produced, for example, by cross-linking a polysaccharide polymer by exposure to a monovalent cation. Other polymers capable of forming hydrogels may be used as disclosed herein, including modified alginate derivatives. In the embodiment where the polymer is crosslinked by contact with a crosslinking agent, the strength of the crosslink may be increased or reduced by adjusting the concentration of the polymer and/or crosslinking agent.

Source of Cells

Cells can be obtained directed from a donor, from cell culture of cells from a donor, or from established cell culture lines. In the preferred embodiment, cells of the same species and preferably immunological profile are obtained by biopsy, either from the patient or a close relative,

which are then grown to confluence in culture using standard conditions and used as needed. If cells that are likely to elicit an immune reaction are used, such as human muscle cells from immunologically distinct individual, then the recipient can be immunosuppressed as needed, for example, using a schedule of steroids and other immunosuppressant drugs such as cyclosporine. However, in the most preferred embodiment, the cells are autologous.

In the preferred embodiments, cells are obtained directly from a donor, washed and implanted directly in combination with the polymeric material. The cells are cultured using techniques known to those skilled in the art of tissue culture. Cells obtained by biopsy are harvested and cultured, passaging as necessary to remove contaminating cells. Isolation of chondrocytes and muscle cells is demonstrated in WO 94/25080, the disclosure of which is incorporated herein.

Cell attachment and viability can be assessed using scanning electron microscopy, histology, and quantitative assessment with radioisotopes. The function of the implanted cells can be determined using a combination of the above-techniques and functional assays. For example, in the case of hepatocytes, *in vivo* liver function studies can be performed by placing a cannula into the recipient's common bile duct. Bile can then be collected in increments. Bile pigments can be analyzed by high pressure liquid chromatography looking for underivatized tetrapyrroles or by thin layer chromatography after being converted to azodipyrroles by reaction with diazotized azodipyrroles ethylanthranilate either with or without treatment with P-glucuronidase. Diconjugated and monoconjugated bilirubin can also be determined by thin layer chromatography after alkalinemethanolysis of conjugated bile pigments. In general, as the number of functioning transplanted hepatocytes increases, the levels of conjugated bilirubin will increase. Simple liver function tests can also be done on blood samples, such as albumin production.

Analogous organ function studies can be conducted using techniques known to those skilled in the art, as required to determine the extent of cell function after implantation. For example, islet cells of the pancreas may be delivered in a similar fashion to that specifically used to implant hepatocytes, to achieve glucose regulation by appropriate secretion of insulin to cure diabetes. Other endocrine tissues can also be implanted. Studies using labelled glucose as well as studies using protein assays can be performed to quantitate cell mass on the polymer scaffolds. These studies of cell mass can then be correlated with cell functional studies to determine what the appropriate cell mass is. In the case of chondrocytes, function is defined as providing appropriate structural support for the surrounding attached tissues.

This technique can be used to provide multiple cell types, including genetically altered cells, within a three-dimensional scaffolding for the efficient transfer of large number of cells and the promotion of transplant engraftment for the purpose of creating a new tissue or tissue equivalent. It can also be used for immunoprotection of cell transplants while a new tissue or tissue equivalent is growing by excluding the host immune system.

Examples of cells which can be implanted as described herein include chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells. As used herein, "organ cells" includes hepatocytes, islet cells, cells of intestinal origin, cells derived from the kidney, and other cells acting primarily to synthesize and secrete, or to metabolize materials.

Addition of Biologically Active Materials to the hydrogel.

The polymeric matrix can be combined with humoral factors to promote cell transplantation and engraftment. For example, the polymeric matrix can be combined with angiogenic factors, antibiotics, antiinflammatories, growth factors, compounds which induce differentiation, and other factors which are known to those skilled in the art of cell culture.

For example, humoral factors could be mixed in a slow-release form with the cell-polymer suspension prior to formation of implant or transplantation. Alternatively, the hydrogel could be modified to bind humoral factors or signal recognition sequences prior to combination with isolated cell suspension.

Polymer Solutions

Polymeric materials which are capable of forming a hydrogel are utilized. The polymer is mixed with cells for implantation into the body and is permitted to crosslink to form a hydrogel matrix containing the cells either before or after implantation in the body. In one embodiment, the polymer forms a hydrogel within the body upon contact with a crosslinking agent. A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is crosslinked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. Naturally occurring and synthetic hydrogel forming polymers, polymer mixtures and copolymers may be utilized as hydrogel precursors.

Examples of materials which can be used to form a hydrogel include modified alginates. Alginate is a carbohydrate polymer isolated from seaweed, which can be crosslinked to form a hydrogel by exposure to a divalent cation such as calcium, as described, for example in WO 94/25080, the disclosure of which is incorporated herein by reference. The modified alginate solution is mixed with the cells to be implanted to form a suspension. Then the suspension is injected directly into a patient prior to crosslinking of the polymer to form the hydrogel containing the cells. The suspension then forms a hydrogel over a short period of time due to the presence *in vivo* of physiological concentrations of calcium ions.

Alginate is ionically crosslinked in the presence of divalent cations, in water, at room temperature, to form a hydrogel matrix. Due to these mild conditions, alginate has been the most commonly used polymer for hybridoma cell encapsulation, as described, for example, in

U.S. Patent No. 4,352,883 to Lim. In the Lim process, an aqueous solution containing the biological materials to be encapsulated is suspended in a solution of a water soluble polymer, the suspension is formed into droplets which are configured into discrete microcapsules by contact with multivalent cations, then the surface of the microcapsules is crosslinked with polyamino acids to form a semipermeable membrane around the encapsulated materials.

Modified alginate derivatives may be synthesized which have an improved ability to form hydrogels. The use of alginate as the starting material is advantageous because it is available from more than one source, and is available in good purity and characterization. As used herein, the term "modified alginates" refers to chemically modified alginates with modified hydrogel properties. Naturally occurring alginate may be chemical modified to produce alginate polymer derivatives that degrade more quickly. For example, alginate may be chemically cleaved to produce smaller blocks of gellable oligosaccharide blocks and a linear copolymer may be formed with another preselected moiety, e.g. lactic acid or ϵ -caprolactone. The resulting polymer includes alginate blocks which permit ionically catalyzed gelling, and oligoester blocks which produce more rapid degradation depending on the synthetic design. Alternatively, alginate polymers may be used, wherein the ratio of mannuronic acid to guluronic acid does not produce a firm gel, which are derivatized with hydrophobic, water-labile chains, e.g., oligomers of ϵ -caprolactone. The hydrophobic interactions induce gelation, until they degrade in the body.

Additionally, polysaccharides which gel by exposure to monovalent cations, including bacterial polysaccharides, such as gellan gum, and plant polysaccharides, such as carrageenans, may be crosslinked to form a hydrogel using methods analogous to those available for the crosslinking of alginates described above. Polysaccharides which gel in the presence of monovalent cations form hydrogels upon exposure, for example, to a solution comprising physiological levels of sodium. Hydrogel precursor

solutions also may be osmotically adjusted with a nonion, such as mannitol, and then injected to form a gel.

Polysaccharides that are very viscous liquids or are thixotropic, and form a gel over time by the slow evolution of structure, are also useful. For example, hyaluronic acid, which forms an injectable gel with a consistency like a hair gel, may be utilized. Modified hyaluronic acid derivatives are particularly useful. As used herein, the term "modified hyaluronic acids" refers to chemically modified hyaluronic acids. Modified hyaluronic acids may be designed and synthesized with preselected chemical modifications to adjust the rate and degree of crosslinking and biodegradation. For example, modified hyaluronic acids may be designed and synthesized which are esterified with a relatively hydrophobic group such as propionic acid or benzylic acid to render the polymer more hydrophobic and gel-forming, or which are grafted with amines to promote electrostatic self-assembly. Modified hyaluronic acids thus may be synthesized which are injectable, in that they flow under stress, but maintain a gel-like structure when not under stress. Hyaluronic acid and hyaluronic derivatives are available from Genzyme, Cambridge, MA and Fidia, Italy.

Other polymeric hydrogel precursors include polyethylene oxide-polypropylene glycol block copolymers such as PluronicTM or TetronicsTM, which are crosslinked by hydrogen bonding and/or by a temperature change, as described in Steinleitner *et al.*, *Obstetrics & Gynecology*, 77:48-52 (1991); and Steinleitner *et al.*, *Fertility and Sterility*, 57:305-308 (1992). Other materials which may be utilized include proteins such as fibrin, collagen and gelatin. Polymer mixtures also may be utilized. For example, a mixture of polyethylene oxide and polyacrylic acid which gels by hydrogen bonding upon mixing may be utilized. In one embodiment, a mixture of a 5% w/w solution of polyacrylic acid with a 5% w/w polyethylene oxide (polyethylene glycol, polyoxyethylene) 100,000 can be combined to form a gel over the course of time, *e.g.*, as quickly as within a few seconds.

Covalently crosslinkable hydrogel precursors also are useful. For example, a water soluble polyamine, such as chitosan, can be cross-linked with a water soluble diisothiocyanate, such as polyethylene glycol diisothiocyanate. The isothiocyanates will react with the amines to form a chemically crosslinked gel. Aldehyde reactions with amines, e.g., with polyethylene glycol dialdehyde also may be utilized. A hydroxylated water soluble polymer also may be utilized.

Alternatively, polymers may be utilized which include substituents which are crosslinked by a radical reaction upon contact with a radical initiator. For example, polymers including ethylenically unsaturated groups which can be photochemically crosslinked may be utilized, as disclosed in WO 93/17669, the disclosure of which is incorporated herein by reference. In this embodiment, water soluble macromers that include at least one water soluble region, a biodegradable region, and at least two free radical-polymerizable regions, are provided. The macromers are polymerized by exposure of the polymerizable regions to free radicals generated, for example, by photosensitive chemicals and or light. Examples of these macromers are PEG-oligolactyl-acrylates, wherein the acrylate groups are polymerized using radical initiating systems, such as an eosin dye, or by brief exposure to ultraviolet or visible light. Additionally, water soluble polymers which include cinnamoyl groups which may be photochemically crosslinked may be utilized, as disclosed in Matsuda *et al.*, *ASAID Trans.*, 38:154-157 (1992).

In general, the polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions. Methods for the synthesis of the other polymers described above are known to those skilled in the art. See, for example Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, E. Goethals, editor (Pergamen Press, Elmsford, NY 1980). Many polymers, such as poly(acrylic acid), are commercially available. Naturally occurring and synthetic polymers may be modified using chemical reactions available in the art and described, for example, in

March, "Advanced Organic Chemistry," 4th Edition, 1992, Wiley-Interscience Publication, New York.

Water soluble polymers with charged side groups may be crosslinked by reacting the polymer with an aqueous solution containing ions of the opposite charge, either cations if the polymer has acidic side groups or anions if the polymer has basic side groups. Examples of cations for cross-linking of the polymers with acidic side groups to form a hydrogel are monovalent cations such as sodium, and multivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, and di-, tri- or tetra-functional organic cations such as alkylammonium salts. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Additionally, the polymers may be crosslinked enzymatically, *e.g.*, fibrin with thrombin.

In the embodiment wherein modified alginates and other anionic polymers that can form hydrogels which are malleable are used to encapsulate cells, the hydrogel is produced by cross-linking the polymer with the appropriate cation, and the strength of the hydrogel bonding increases with either increasing concentrations of cations or of polymer. Concentrations from as low as 0.001 M have been shown to cross-link alginate. Higher concentrations are limited by the toxicity of the salt.

The preferred anions for cross-linking of the polymers to form a hydrogel are monovalent, divalent or trivalent anions such as low molecular weight dicarboxylic acids, for example, terephthalic acid, sulfate ions and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels and membranes, as described with respect to cations.

A variety of polycations can be used to complex and thereby stabilize the polymer hydrogel into a semi-permeable surface membrane. Examples of materials that can be used include polymers having basic

reactive groups such as amine or imine groups, having a preferred molecular weight between 3,000 and 100,000, such as polyethylenimine and polylysine. These are commercially available. One polycation is poly(L-lysine); examples of synthetic polyamines are: polyethylenimine, poly(vinylamine), and poly(allyl amine). There are also natural polycations such as the polysaccharide, chitosan.

Polyanions that can be used to form a semi-permeable membrane by reaction with basic surface groups on the polymer hydrogel include polymers and copolymers of acrylic acid, methacrylic acid, and other derivatives of acrylic acid, polymers with pendant SO_3H groups such as sulfonated polystyrene, and polystyrene with carboxylic acid groups.

Cell Suspensions

Preferably the polymer is dissolved in an aqueous solution, preferably a 0.1 M potassium phosphate solution, at physiological pH, to a concentration forming a polymeric hydrogel, for example, for modified alginate, of between 0.5 to 2% by weight, e.g., 1%, modified alginate. The isolated cells are suspended in the polymer solution to a concentration of between 1 and 50 million cells/ml, most preferably between 10 and 20 million cells/ml.

Methods of Implantation

The techniques described herein can be used for delivery of many different cell types to achieve different tissue structures. In the preferred embodiment, the cells are mixed with the polymer solution and injected directly into a site where it is desired to implant the cells, prior to crosslinking of the polymer to form the hydrogel matrix. However, the matrix may also be molded and implanted in one or more different areas of the body to suit a particular application. This application is particularly relevant where a specific structural design is desired or where the area into which the cells are to be implanted lacks specific structure or support to facilitate growth and proliferation of the cells.

The site, or sites, where cells are to be implanted is determined based on individual need, as is the requisite number of cells. For cells

having organ function, for example, hepatocytes or islet cells, the mixture can be injected into the mesentery, subcutaneous tissue, retroperitoneum, properitoneal space, and intramuscular space. For formation of cartilage, the cells are injected into the site where cartilage formation is desired.

5 One could also apply an external mold to shape the injected solution. Additionally, by controlling the rate of polymerization, it is possible to mold the cell-hydrogel injected implant like one would mold clay. Alternatively, the mixture can be injected into a mold, the hydrogel allowed to harden, then the material implanted.

10 The suspension can be injected via a syringe and needle directly into a specific area wherever a bulking agent is desired, i.e., a soft tissue deformity such as that seen with areas of muscle atrophy due to congenital or acquired diseases or secondary to trauma, burns, and the like. An example of this would be the injection of the suspension in the upper
15 torso of a patient with muscular atrophy secondary to nerve damage.

The suspension can also be injected as a bulking agent for hard tissue defects, such as bone or cartilage defects, either congenital or acquired disease states, or secondary to trauma, burns, or the like. An example of this would be an injection into the area surrounding the skull
20 where a bony deformity exists secondary to trauma. The injunction in these instances can be made directly into the needed area with the use of a needle and syringe under local or general anesthesia.

The suspension could also be injected percutaneously by direct palpation, such as by placing a needle inside the vas deferens and
25 occluding the same with the injected bulking substance, thus rendering the patient infertile. The suspension could also be injected through a catheter or needle with fluoroscopic, sonographic, computed tomography, magnetic resonance imaging or other type of radiologic guidance. This would allow for placement or injection of this substance either by vascular
30 access or percutaneous access to specific organs or other tissue regions in the body, wherever a bulking agent would be required.

Further, this substance could be injected through a laparoscope or thoracoscope to any intraperitoneal or extraperitoneal or thoracic organ. For example, the suspension could be injected in the region of the gastro-esophageal junction for the correcting of gastroesophageal reflux. This could be performed either with a thoracoscope injecting the substance in the esophageal portion of the gastroesophageal region, or via a laparoscope by injecting the substance in the gastric portion of the gastroesophageal region, or by a combined approach.

Vesicoureteral reflux is one of the most common congenital defects in children, affecting approximately 1% of the population. Although all patients do not require surgical treatment, it is still one of the most common procedure performed in children. Over 600 ureteral reimplants are performed yearly at Children's Hospital in Boston, Massachusetts. This translates to an approximately saving of 3600 inpatient hospital days per year at this institution alone, if the endoscopic treatment described herein is used instead of open surgery.

In addition to its use for the endoscopic treatment of reflux, the system of injectable autologous muscle cell may also be applicable for the treatment of other medical conditions, such as urinary and rectal incontinence, dysphonia, plastic reconstruction, and wherever an injectable permanent biocompatible material is needed. Methods for using an injectable polymer for delivering isolated cells via injection are described for example in WO 94/25080.

Improved injectable biocompatible polymers are disclosed herein which are useful for example as a delivery vehicle for muscle cells or chondrocytes in the treatment of reflux and incontinence. In one exemplary embodiment, a biopsy is obtained under anesthesia from a patient with vesicoureteral reflux, the isolated cells are mixed with a polymer capable of crosslinking to form a hydrogel, and the cell-polymer solution is injected endoscopically in the sub-ureteral region to correct reflux. The time to solidification of the polymer-cell solution may be manipulated by varying the concentration of the crosslinking agent as well

as the temperature at which the cells are added to the polymer. The use of autologous cells precludes an immunologic reaction. Solidification of the polymer impedes its migration until after it is degraded. The suspension can be injected through a cystoscopic needle, having direct visual access with a cystoscope to the area of interest, such as for the treatment of vesico-ureteral reflux or urinary incontinence.

In addition to the use of the cell-polymer suspension for the treatment of reflux and incontinence, the suspension can also be applied to reconstructive surgery, as well as its application anywhere in the human body where a biocompatible permanent injectable material is necessary. The suspension can be injected endoscopically, for example through a laryngoscope for injection into the vocal chords for the treatment of dysphonia, or through a hysteroscope for injection into the fallopian tubes as a method of rendering the patient infertile, or through a proctoscope, for injection of the substance in the perirectal sphincter area, thereby increasing the resistance in the sphincter area and rendering the patient continent of stool.

This technology can be used for other purposes. For example, custom-molded cell implants can be used to reconstruct three dimensional tissue defects, e.g., molds of human ears could be created and a chondrocyte-hydrogel replica could be fashioned and implanted to reconstruct a missing ear. Cells can also be transplanted in the form of a three-dimensional structure which could be delivered via injection.

The teachings of the cited publications are indicative of the level of skill and the general knowledge of those skilled in the art. To the extent necessary, the publications are specifically incorporated herein by reference.

I claim:

1. A method for implanting tissue into an animal comprising mixing dissociated cells with a solution of a biocompatible polymer capable of crosslinking to form a hydrogel;
implanting the mixture into the animal; and
permitting the polymer to crosslink and form a hydrogel matrix having the cells dispersed therein.
2. The method of claim 1 wherein the hydrogel matrix containing the cells forms before implantation.
3. The method of claim 1 wherein the mixture of the polymer and the cells is injected into the animal as a cell suspension; and
wherein the hydrogel matrix containing the cells forms after the mixture is injected.
4. The method of claim 1 wherein the polymer is a mixture of polyethylene oxide and polyacrylic acid.
5. The method of claim 1 wherein the polymer is selected from the group consisting of a modified aliginate, a modified hyaluronic acid and gelatin.
6. The method of claim 1 wherein the hydrogel is formed by exposure of the polymer to a crosslinking agent selected from the group consisting of ions, pH changes, radical initiators, enzymes, and temperature changes.
7. The method of claim 1 wherein the polymer is a polysaccharide which forms a hydrogel upon exposure to a monovalent cation.
8. The method of claim 7 wherein the polysaccharide is selected from the group consisting of a gellan gum and a carrageenan.
9. The method of claim 7 wherein the cation is sodium.

10. The method of claim 6 wherein the polymer comprises substituents capable of crosslinking by a radical reaction in the presence of a radical initiator to form a hydrogel, and wherein the polymer is contacted with the radical initiator thereby to covalently crosslink the polymer by a radical reaction and form the hydrogel.

11. The method of claim 10 wherein the radical initiator is selected from the group consisting of a dye, ultraviolet light and visible light.

12. The method of claim 1 wherein the polymer comprises substituents capable of covalently reacting with a crosslinking agent, and wherein the polymer is contacted with the crosslinking agent thereby to covalently crosslink the polymer and form the hydrogel.

13. The method of claim 1 wherein the cells are selected from the group consisting of chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells.

14. The method of claim 2 wherein the hydrogel is molded to form a specific shape prior to implantation.

15. The method of claim 3 wherein the hydrogel is molded to form a specific shape after mixing with the cells and being implanted into the animal.

16. The method of claim 1 for treating vesicoureteral reflux.

17. The method of claim 1 for treating incontinence.

18. The method of claim 1 for treating a defect in the thoracic region.

19. The method of claim 1 for treating the upper gastrointestinal tract.

20. A composition for implanting cells in an animal comprising: a mixture of dissociated cells and a solution of a biocompatible polymer capable of crosslinking to form a hydrogel matrix having the cells dispersed therein.

21. The composition of claim 20 wherein the polymer is capable of forming the hydrogel matrix having cells disposed therein prior to implantation in the animal.

22. The composition of claim 20 wherein the polymer is capable of forming the hydrogel matrix having cells disposed therein after implantation of the mixture in the animal by injection.

23. The composition of claim 20 wherein the polymer is selected from the group consisting of a modified aliginate, a modified hyaluronic acid and gelatin.

24. The composition of claim 20 wherein the hydrogel is formed by exposure of the polymer to a crosslinking agent selected from the group consisting of ions, pH changes, radical initiators, enzymes, and temperature changes.

25. The composition of claim 20 wherein the polymer is a polysaccharide which forms a hydrogel upon exposure to a monovalent cation.

26. The composition of claim 25 wherein the polysaccharide is selected from the group consisting of a gellan gum and a carrageenan.

27. The composition of claim 25 wherein the cation is sodium.

28. The composition of claim 24 wherein the polymer comprises substituents capable of crosslinking by a radical reaction in the presence of a radical initiator to form a hydrogel.

29. The composition of claim 20 wherein the polymer comprises substituents capable of covalently reacting with a crosslinking agent, thereby to covalently crosslink the polymer and form the hydrogel.

30. The composition of claim 20 wherein the cells are selected from the group consisting of chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO,A,94 25080 (MASSACHUSETTS INST TECHNOLOGY ;CHILDRENS MEDICAL CENTER (US)) 10 November 1994 cited in the application see claims --- | 1-30 |
| X | US,A,4 846 835 (GRANDE DANIEL A) 11 July 1989 see the whole document --- | 1-3,13 |
| P,X | WO,A,95 26761 (COLLAGEN CORP) 12 October 1995 see claims --- | 1-3, 20-22 |
| P,X | WO,A,96 03160 (CHILDRENS MEDICAL CENTER ;GEN HOSPITAL CORP (US)) 8 February 1996 see claims --- | 1-3,13, 16-19,30 |
| | -/- | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'A' document member of the same patent family

Date of the actual completion of the international search

10 October 1996

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ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PC./US 96/09065

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| A | WO,A,88 03785 (VACANTI JOSEPH P ;LANGER ROBERT S (US)) 2 June 1988 see claims --- | 1-30 |
| A | WO,A,91 01720 (SCHLAMEUS HERMAN WADE ;FOX WILLIAM CASEY (US); MANGOLD DONALD JACO) 21 February 1991 see claims ----- | 1-30 |

INTERNATIONAL SEARCH REPORT

I national application No.

PCT/US 96/09065

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : although claims 1-19 are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PC./US 96/09065

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
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| US-A-4846835 | 11-07-89 | NONE | |
| WO-A-9526761 | 12-10-95 | AU-A- 2202995 | 23-10-95 |
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| WO-A-9101720 | 21-02-91 | US-A- 5294446 | 15-03-94 |